THE DISTRIBUTION OF THE SPORES OF B. BOTULINUS IN THE UNITED STATES. IV *

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It has been customary to consider California an endemic focus of botulism and the prevention of this intoxication a problem confined to the Pacific Coast States. Such a view is generally held in the East and Middle West, and statements have from time to time been issued which tend to absolve the territories east of the Rocky mountains and place the odium of botulism squarely on California. In a recent paper [1] it has been shown that this apparent prevalence of botulism in the last named state is in all probability due to the interest this disease has received at the hands of a number of workers, as a result of which data have been unearthed and analyzed which have thus far not focused attention in the other states of the Union. Furthermore, an unbiased observer will have noted that botulism is an acquisition of recent years and is closely associated with the use of home or commercially canned food products. During the war, economic reasons prompted the adoption of home canning procedures, while later prosperity on one side and domestic labor difficulties on the other led to the extensive consumption of commercially preserved, "ready to serve" foods of plant and animal origin. Since 1912, eighty-seven outbreaks of human and animal botulism have been caused by food products grown and packed in 20 states, exclusive of California. In this connection the following states and outbreaks should be mentioned: Colorado (3 human and 3 fowl), District of Columbia (1 fowl), Florida (1 human), Idaho (3 human), Illinois (1 human, 1 fowl and 1 horse), Indiana (1 human and 1 fowl), Iowa (1 human), Kentucky (1 mule), Main (1 human), Massachusetts (3 human), New Jersey (1 human and fowl), New York (4 human), Nevada (5 fowl), Ohio (2 human), Oregon (4 human and 2 fowl), Pennsylvania (1 human), Texas (1 human), Washington (14 human and 23 chicken), Wisconsin (1 human) and Wyoming (1 human).

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[1] The Epidemiology of Botulism, to be published in a Bulletin of the U. S. Public Health Service.

This summary permits one conclusion: The spores of B. botulinus must be widely distributed in the United States. In order to confirm this, however, an examination of soil, vegetable and manure samples procured in every state in the Union was undertaken. It is proposed in this paper to summarize these studies. It will be shown that no territory within the boundaries of the United States can justifiably claim immunity from B. botulinus. Absence of human or animal botulism from a number of states cannot be attributed to the non-existence of the spores of the causative organism in the soil, but is probably the result of certain other factors, which will be discussed in the analysis of the 1,538 specimens secured for this investigation.

Organization for Collecting and Culture of the Samples

The same principles as outlined in the preceding paper dealing with the collection of field specimens in California have been followed in this study. It is self-evident that the cooperation of a number of agencies had to be enlisted in order to make a complete and comparative survey. Dr. H. M. Loomis, Director of Inspection of the National Canners Association, through his field staff in Ohio, Michigan, Illinois, Colorado, Wisconsin, etc., obtained and forwarded valuable samples from the cultivated fields supplying the raw material to important canneries in these states. Dr. C. M. Haring, Director, Agricultural Experiment Station, University of California, solicited and secured the cooperation of a large number of agricultural experiment stations. Sets of at least 20 to 30 specimens were procured through these agencies, namely, one earth sample each from a corral which had been used for cattle during the past year, a pig pen, a poultry yard, a field used for corn, for grain, for clover or as pasture during the past year, several samples of cornstalks, of moldy straw, of decaying vegetation, of bean pods and stalks, of peas, of fresh greens, such as beet tops, spinach, etc., and of roots, such as carrots, beets, etc. These specimens proved valuable from a comparative standpoint and furnished a number of important facts. The study dealing with the distribution of B. botulinus in the state of Washington was based on specimens collected by Dr. J. C. Geiger during his survey on botulismus made in the spring of 1921 at Yakima, Tieton, Toppenish and Yelm. State boards of health, universities and colleges from neighboring states—in particular, Nevada, Utah, Oregon, Arizona and Colorado—frequently submitted samples for examination and rendered valuable assistance in this respect. In order to verify the conclusions reached for California that B. botulinus is an organism of the virgin soil, Dr. J. C. Geiger collected, in September and October, 1921, from 25 to 50 earth samples each from the Glacier and Mt. Rainier National Parks, from the Ozark Mountains in Arkansas, from the foothills near Alexandria in Louisiana, from the foothills near Bath in Maine, from the Blue Ridge Mountains near Washington, D. C., from Mount Baker near Saranac Lake in New York and from the Allegheny Mountains in West Virginia.

It is not only a duty, but a great pleasure, to acknowledge the assistance rendered by the various laboratories, experiment stations, universities and individual staff members who devoted time and energy to the collection of specimens and, through their efforts, enabled the Foundation to make a fairly complete and comparative survey.

The samples were collected in containers which had been sterilized by dry heat at a temperature of not less than 170 C. for 2 hours, or by live steam at a pressure of at least 15 pounds for 30 minutes. When received in the laboratory, extreme precautions were taken to avoid mixture or contamination. Mass cultures were prepared according to the procedures outlined in the first paper of this series; in fact, all cultures were made with heated specimens in beef heart peptic digest liver broth and incubated for 10 days. Crude and unfiltered cultures were tested on guinea-pigs either by subcutaneous inoculation or feeding. The nature and type of the toxin were identified on the same species of animal. In a number of instances, pure cultures were isolated from the enrichment cultures.

EXPERIMENTAL DATA

The character of the specimens, the cultural results and the epidemiologic facts thus far determined are reported separately for each state. A geographic, alphabetical presentation has again been chosen to facilitate subsequent reference in the discussion.

Alabama.—In August, 1921, Professor Wright A. Gardner, Alabama Polytechnic Institute, Auburn, Alabama, forwarded 16 specimens: soil from hog pen (2), cow lot (2), oat field (1), clover patch (1), chicken yard (1), corn field (1), vegetable garden (2), onions (1), green corn stalks (1), old corn stalks (1), moldy hay (1), lima beans (1), and decaying vegetables (1). B. botulinus type A was found in the culture prepared with the green corn stalks.

Summary: 10 cultivated soils or animal corrals, 6 vegetables and feeds. Total: 16 with 1, or 6.2%, positive culture; 1 type A.

Arizona.—One human outbreak at Florence, commercially canned beets grown and packed in Ohio.

Miss Jane Rider, Director, State Laboratory, University of Arizona, Tucson, submitted, in February, 1921, one sample of "filaree" hay collected in the southern part of the state and suspected of causing forage poisoning in horses. The culture proved nontoxic.

Arkansas.—In May, 1921, Dr. William L. Bleecker, Bacteriologist, University of Arkansas, College of Agriculture, Fayetteville, furnished 21 specimens: soil from pasture (1), cow lot (1), clover field (1), grain field (1), corn field (1), hog lot (2), poultry yard (1), cow peas (1), radishes (2), spinach (1), soja beans (1), lettuce (1), cornstalk (1), corn fodder (1), rotted straw (1), decaying leaves (2), moldy straw (1), and moldy silage (1).

The rotted straw gave a culture containing the toxin of B. botulinus type B, while the soja beans, decayed leaves and the soil from the hog pen produced toxic cultures, but the nature of the toxin could not be determined by a toxin-antitoxin test.

In November, 1921, Dr. J. C. Geiger collected 20 samples of virgin (?) soil from the Ozark Mountains, Government Reservation, Hot Springs, Ark. Two cultures were weakly toxic, 1 contained B. tetani and 4 produced "malignant edema."

Summary: 8 cultivated soils or animal corrals, 1 weak toxin, 20 virgin soil, 2 weak toxin, 13 feeds, vegetables, 1 type B; 2 weak toxin. Total: 41 with 1 (6), or 2.4 (14.8) % positive cultures.

Colorado.—Three human outbreaks (2 caused by commercially canned string beans or spinach grown and packed in the state of Kansas; 1 home canned beets grown and packed near Pueblo); 3 chicken outbreaks due to home canned vegetables (2 corn, Fort Collins, and 1 string beans, Lyons).

Mr. R. S. Hiltner, Director, Colorado Inspection Service, of the National Canners Association, Denver, procured, in December, 1920, from Brighton (4,970 ft.) and Longmont (4,935 ft.), Colorado, a total of 27 samples consisting of soils (12), tomato plants and roots (4), bean plants, vines and roots (8), pumpkin and cucumber vines and roots (3) were cultures; one culture, prepared from a wax bean plant (East of Longmont), was toxic, but the nature of the toxin could not be determined by a toxin-antitoxin test.

In December, 1921, Inspector M. M. Allison collected 3 sets of soil and plant remnants from farms near Fowler, Colo., in the Arkansas Valley, about 40 miles east of Pueblo. The farms were irrigated by ditches from the Arkansas River. Nine cultures, prepared from soils (3), tomato stems, leaves and roots (2), bean stalks and roots (2) and cucumber vines and roots (2), gave the following results:

Soil, tomato field
Soil, bean field
Bean stalks
Bean roots
} B. botulinus, type A.

Tomato roots and vines from cucumber fields produced weakly toxic cultures.

Dr. I. E. Newsom, Department of Pathology, Colorado Agricultural College and Experiment Station, Fort Collins, submitted for examination a specimen of dried crop content of a chicken (Lyons) and 5 samples of moldy alfalfa and dry beet pulp (feed lot, Ault sugar farm near Greeley). The crop specimen contained B. botulinus, type A (for details see Chicken Report 41). In April, 1922, a specimen of home canned corn and the crop content of a chicken, which contained B. botulinus, type A, was received from the same laboratory.

In Sept., 1921, Drs. Oscar I. Kron and F. M. Hayes collected 14 virgin soil samples from Pike's Peak (at an elevation of 11,000 ft.), 5 samples from Cheyenne Canyon, 4 from Mushroom Park, Garden of the Gods, and 4 from the Cave of the Winds. The cultural findings were:

Pike's Peak, 4—B. botulinus, type A; 2 weak toxins.

Cheyenne Canyon, 1—B. botulinus, type A.

Mushroom Park, 1—B. botulinus, type A.

Cave of the Winds, 1—B. botulinus, type A.

Summary: 32 cultivated soils and its products; 1 weak toxin; 9 irrigated soils and its products; 4 type A; 2 weak toxins; 27 virgin mountain soil; 7 type A; 2 weak toxins.

Total: 68 samples with 11 (16), or 16.1 (25.0) % positive cultures.

One crop specimen (home canned string beans) type A; 1 home canned corn and 1 crop specimen, type A.

Connecticut.—In Sept., 1921, Mr. C. J. Mason, Instructor in Bacteriology, Connecticut Agricultural College, Storrs, collected and submitted for examination, 9 samples of soil (poultry yard, pig pasture [2], grass field [2], clover field, cattle corral, pig pen, corn field) and 15 vegetable specimens (beets, potatoes, carrots [2], peas, musty straw, beet greens, corn stalks, turnip tops, decayed tomatoes, cucumbers, bean stalks, bean pods, musty hay, mangel tops). The beets furnished a type A, B. botulinus, while the potatoes, musty straw, corn stalks, decayed bean stalks and tomatoes gave weakly toxic cultures.

Summary: 9 cultivated or manured soils, 15 vegetables, feed, etc.—1 type A, 5 weak toxins. Total: 24 samples with 4.1 (25) % positive cultures.

Delaware.—Mr. F. C. Blanck, Director of Inspection, National Canners Association, Easton, Md., collected and forwarded, in December, 1920, from different points in Delaware, 6 samples of soil (lima bean, tomato, red pepper,

string bean, bush bean and sweet potato fields) and 12 specimens of leaves and roots from the fields mentioned. The following cultures were toxic:

Soil, husks and leaves from string bean field, 2 B. botulinus, type B.

Soil, tops and leaves from tomato field; 2 B. botulinus, type B.

Lima beans and bush bean husks, weak toxins.

Summary: 6 cultivated soil, 2 type B. 12 plant products, 2 type B and 2 weak toxins.

Total: 18 samples with 4 (16), or 22.2 (33.3) % positive cultures.

District of Columbia.—One chicken outbreak, due to corn, no earth samples.

Florida.—One human outbreak at Tampa due to home preserved ham.

In November, 1921, Dr. J. C. Geiger collected, near Miami, 20 samples of unfertilized soil. One culture contained a weak toxin. At the same time, Dr. W. G. W. Ells, Assistant Plant Pathologist, Agricultural Experiment Station, University of Florida, Gainesville, collected and forwarded 25 duplicate samples of soils and decayed plants from the vicinity of the experiment station. Cultures were made from soil from cattle corral, horse corral, cane field, citrus orchard, garden, corn field, hog lot, chicken run, muck soil, peat, clay, barren field, dirt road, edge of pond, from under yellow pine tree, shrub land, potato land, Bermuda sod, meadow (Natal grass), cherry laurel hedge, virgin soil from hammock, oak and hickory, yellow pine land and large oak land and decomposed roots and leaves. Eight cultures were positive, namely:

Decomposed roots and leaves, 2—B. botulinus, type B.

Soil from citrus orchard, 1—B. botulinus, type B.

Soil from meadow, Natal grass, 1—B. botulinus, type B.

Muck soil, 1—B. botulinus, type B.

Soil from edge of a pond, 1—B. botulinus, type B.

Soil from ordinary, poor blackjack shrub land, 1—B. botulinus, type B.

Soil from cane field, 1—B. botulinus, type A.

Summary: 43 soils from diverse sources, 5 type B, B. botulinus, 1 type A, 1 weak toxin; 2 decomposed plants, 2 type B.; 45 with 8, or 17.7% positive cultures.

Georgia.—In May, 1921, Dr. B. B. Higgins, Botanist, Georgia Experiment Station, Experiment, Ga., collected and submitted for examination 20 samples of soils and its products: soil from pasture, cow lot, corn stubble, clover land, chicken yard, hog lot and virgin soil, moldy hay (2), bean pods and stalks, beans and vine, cabbage (2), peas, cornstalk, onions, daffodil bulbs, turnips, English garden peas, beet root. Five cultures gave relatively strong toxins:

Moldy hay, 1—B. botulinus, type B.

Bean pods and stalks, 1—B. botulinus, type B.

Beans and vines, 1—B. botulinus, type B.

Decayed cabbage, 1—B. botulinus, type B.

Turnips, 1—B. botulinus, type B.

Summary: 7 soil samples, all negative; 13 vegetables and feeds, 5 type B.

Total: 20 samples with 5, or 25%, positive cultures.

Idaho.—Three human outbreaks (1 locally grown and home canned asparagus, Boise, 1 locally grown and home canned greens and turnip tops, Cambridge, and 1 locally grown and home canned corn, Weiser).

In November, 1921, Professor William M. Gibbs, head of the Department of Bacteriology, College of Agriculture and Agricultural Experiment Station, University of Idaho, Moscow, collected and forwarded for examination 32 samples of soils and vegetables and feeds: soil from turnip field, chicken yard, cattle pasture, manure, pea field, corn field, sheep corral, cattle corral (2), pig

pen, orchard, grain lot, subsoil excavation, virgin soil (3), turnip tops (2), grass from pasture, corn stalk, peas and roots, corn, decomposed silage, larch leaves (Arboretum), cabbage, apples, pears and cucumbers rotting on ground, beet tops, beets dug from ground, chard and parsnips dug from ground.

Three cultures were highly toxic, while 3 produced symptoms, but could not be identified. The following samples furnished these cultures:

Virgin soil under larch growth, B. botulinus, type A.

Cucumber rotting on ground, B. botulinus, type A (isolated).

Beet tops, beets still growing, B. botulinus, type B.

Soil from turnip field, cornstalk and beets, dug from ground gave weak toxins.

In February, 1922, Dr. W. R. Hamilton, Weiser, sent a jar of locally grown and packed home canned corn belonging to the same lot, which caused the death of one child at Weiser, Report 99. The corn was toxic and B. botulinus, type A, was isolated.

Summary: 15 soil samples, 1 virgin soil, type A; 18 vegetables and feeds and home canned plant products, 2 type A and 1 type B, 3 weak toxins.

Total: 33 samples with 4 (7), or 12.1 (21.2)%, positive cultures.

Illinois.—One incompletely proved human outbreak at Chicago, one chicken outbreak, due to garbage, one outbreak among horses caused by ensilage.

The Hoopeston Canning Company submitted, in December, 1920, 2 samples of soil collected from heavily manured corn fields and 2 specimens of sweet corn roots; 8 cultures were prepared and one of each soil specimen contained a weak toxin, producing symptoms and death in 10 days.

The Gibson Canning Company, Gibson City, furnished, in December, 1920, one soil sample and 5 specimens of sweet corn stalks, husks, leaves, etc. One culture prepared with the corn stalk contained a toxin which was fatal to a guinea-pig on the twenty-sixth day.

Mr. David M. Morgan of Mound City forwarded 5 samples of soil (sweet corn, tomato, sweet potato, hominy, corn and pumpkin fields) and 10 specimens of roots, leaves, vines, etc., collected from the same areas in December, 1920. Ten specimens of the cultures prepared with the sweet corn and sweet potato leaves contained B. botulinus, type B.

The Interrieden Canning Company, Grayslake, forwarded 5 soil specimens and 7 samples of corn leaves, husks, pea vines, roots and leaves collected on their farms, in December, 1920. The cultures prepared with these specimens were all nontoxic.

The George E. Stocking Canning Factory, Rochelle, supplied 2 specimens of soil derived from a pea field together with some leaves of a well cured stack. The samples furnished nontoxic cultures.

Summary: 14 soils from pea, corn, tomato, etc., fields, 2 weak toxins; 25 leaves, husks, vines, etc., 2 type B, B. botulinus, and 1 weak toxin.

Total: 39 samples with 2 (5), or 5.1 (12.8) %, positive cultures.

Indiana.—Two human outbreaks (one due to locally grown and home canned string beans and one due to commercially packed spinach from California, at Kendallville).

Mr. John P. Street, Director of Indiana Inspection Service, National Canners Association, Indianapolis, procured 1 sample of soil and 2 specimens of roots and stems from a tomato field at Paoli, and 3 similar specimens from Red Key. These samples furnished nontoxic cultures.

Dr. L. P. Doyle, Purdue University, Agricultural Experiment Station, Lafayette, collected and submitted for examination 7 samples of soil from cattle pen, pig pen, poultry yard, corn field, wheat field, clover field and

pasture, and 15 specimens of vegetables and plants: corn stalk, decaying vegetables (2), straw, moldy straw, beanstalks, bean pods (2), pea vine, swiss chard, beet top, turnips, beets, carrots and radishes. The following cultures were toxic:

Soil corn field, 1—B. botulinus, type B.
Soil wheat field, 1—B. botulinus, type B.
Soil clover field, 1—B. botulinus, type B.
Soil pasture, 1—weak toxin and B. tetani.
Bean stalks and pods, 2—B. botulinus, type B, 1 weak toxin.
Radishes, 1—weak toxin.
Swiss chard, B. tetani.

Summary: 9 soils from cultivated fields and pasture, 3 type B, 1 weak toxin and B. tetani; 19 vegetables and feeds, 2 type B, 2 weak toxin and B. tetani.

Total: 28 samples with 5 (8), or 17.8 (28.4) %, positive cultures.

Iowa.—One unproved human outbreak, due to home prepared ham and salted pork, at Sioux Rapids.

Mr. William H. Harrison, Director of the Iowa Inspection Service, National Canners Association, collected and forwarded, in December, 1920, one sample of soil and one specimen each of corn leaves, husks and roots on a farm near Altoona. Similar sets of specimens were taken at Grimes (about 14 miles northwest of DesMoines) and at Vinton.

Dr. Charles Murray, Division of Veterinary Medicine, Iowa State College, Ames, collected, in September, 1921, and forwarded for study 8 samples of soil (hog yard, horse corral, cattle corral, poultry yard, corn field, clover field, oat field, pasture) and 14 specimens of vegetables and feeds: oats (2), beets, carrots, turnips, beans and pods, decayed straw, cabbage, radish roots and tops, moldy hay, corn stalks, decayed vegetables (2).

One sample of oats gave a culture containing B. botulinus type A, while those prepared from the radish roots and carrots were weakly toxic.

Summary: 11 cultivated soils, all negative; 23 vegetables and feeds, 1 type A, 2 weak toxins.

Total: 34 samples with 1 (3), or 2.9 (5.8) %, positive.

Kansas.—Mr. H. R. Baker, Department of Bacteriology, Kansas State Agricultural College, Manhattan, collected and forwarded, in May, 1921, 10 samples of soil (horse corral [2], cow pasture, cow corral, pig pen, sheep corral, poultry yard, grain field, clover field and corn field fertilized with sheep manure) and 20 specimens of vegetables and feeds: bottom of a Sudan grass straw stack, decayed straw (2), corn stalks, rotted, frost bitten alfalfa, young green mangels, young turnips, decayed leaves in barnyard, spinach, straw, beets, string beans, carrots, radishes, beet tops, pea stalks, decayed vegetation, and bean stalks and lettuce (2).

Beets bought on the market, B. botulinus, type A.
String beans bought on the market, B. botulinus, type A.

Summary: 10 cultivated or manured soils, all negative; 20 vegetables and feeds, 2 type A.

Total: 30 samples with 2, or 6.6%, positive cultures.

Kentucky.—One outbreak among mules, due to ensilage prepared in Carroll County.

Dr. A. J. Steiner, Department of Veterinary Science, Agricultural Experiment Station of the College of Agriculture, University of Kentucky, Lexington, collected and submitted, in June, 1921, 7 samples of soil (hog pen, dairy pasture, manure and soil from cow run, chicken run, corn field [2], rye field)

and ten specimens of vegetables and feeds: asparagus roots, potato, wheat heads, lettuce, spinach, onions, beets, corn stalks, soja beans and radishes. The following specimens furnished highly toxic cultures:

Soil from hog pen on college farm (has been used as such for many years, B. botulinus, type B.

Soil from dairy pasture, B. botulinus, type B.

Soil from field that contained clover last year, corn this year, B. botulinus, type B.

Soil from plot which has been planted in corn for the last seven years, B. botulinus, type B.

Radishes, onions, beets and lettuce from Dr. D.'s garden, 4 B. botulinus, type B.

Potato from College farm garden, B. botulinus, type B.

Wheat heads from College farm, B. botulinus, type B.

Corn stalks from Experiment Station Farm, B. botulinus, type A.

Soja beans from Experiment Station Farm, B. botulinus, type B.

Summary: 7 cultivated or manured soils, 4 type B; 10 vegetables and feeds, 7 type B, 1 type A.

Total: 17 samples with 12, or 70.5%, positive cultures.

Louisiana.—In March, 1921, Dr. Harry Morris, Bacteriologist, College of Agriculture and Agricultural Experiment Station, Louisiana State University, Baton Rouge, collected around the University Campus and Experiment Station 8 samples of soil (cattle lot, hog lot, chicken yard, oat field, sugar cane field, pasture, corn field, alfalfa field) and 20 specimens of plant products: mustard, beets, decaying rice straw (2), moldy soja bean hay, lettuce, pea pods and stalks, soja beans, decaying onions, beet tops, green beans, decaying sweet potatoes, decaying leaves, carrot tops, carrots, decaying cabbage leaves, corn stalks (2), husks and corn. The following cultures were toxic:

Corn stalks, B. botulinus, type A.

Moldy soja bean hay, B. botulinus, type A.

Lettuce, beet tops, green beans and corn furnished weakly toxic cultures.

In November, 1921, Dr. J. C. Geiger collected in the foothills 5 miles north of Alexandria, 20 samples of soil from cut over timberland used for grazing purposes. The cultures were all nontoxic.

Summary: 8 cultivated soils, all negative; 20 timberland, used for grazing, all negative; 20 vegetables and feeds, 2 type A, 4 weak toxins.

Total: 48 samples with 2 (6), or 4.1 (12.5) %, positive cultures.

Maine.—One human outbreak due to commercially prepared ham at a summer resort.

Miss F. L. Chandler, Department of Bacteriology and Veterinary Science, University of Maine, Orono, collected and forwarded, in September, 1921, 7 samples of soil (corn field, clover field, hen yard, cow yard, pig pen, pasture, grain field) and 14 specimens of vegetables and plants (cucumbers, lettuce, decaying vegetables, tomatoes, cabbage, beets and tops, beans, carrots, rape, corn stalks, moldy hay, turnips, spoiled silage, pea pods and stalks). The following cultures were highly toxic:

Lettuce, cabbage, moldy hay, pea pods and stalks, 4 B. botulinus, type A.

Beans and turnips, 2 B. botulinus, type B.

In October, 1921, Dr. J. C. Geiger collected from the foothills (uncultivated section) near Bath, Lewiston, Augusta and Poland Springs 20 samples of virgin forest soil. Four cultures made from red soil were highly toxic and contained B. botulinus, type A.

Summary: 7 cultivated soil, all negative; 20 virgin forest soil, 4 type A; 14 vegetables and feeds, 4 type A, 2 type B.

Total: 41 samples with 10, or 24.3%, positive cultures.

Maryland.—Mr. F. C. Blanck, Director of Inspection, National Canners Association, Easton, collected and forwarded, in December, 1920, 2 samples of soil (tomato and corn fields) and 4 specimens of stems and roots procured from the same fields. The cultures prepared from the soil of the corn field contained B. botulinus, type A, while those made with soil of the tomato field and tomato roots were not sufficiently toxic to permit identification.

In February, 1921, cultures were made of 4 samples of hog manure obtained at Perryville, with negative results.

Dr. J. C. Geiger collected 20 samples of virgin soil about 40 to 50 miles from Washington, D. C., in the foothill region of the Blue Ridge Mountains. Eleven cultures contained B. botulinus, type B, 2 type A and B, and 1 B. botulinus, type B, and B. tetani.

Summary: 2 cultivated soil, 1 type A, 1 weak toxin; 20 virgin soil, 11 type B, 2 type A and B, 1 B. tetani and B. botulinus; 4 vegetables, 1 weak toxin; 4 hog manure, all negative.

Total: 30 samples with 15 (17), or 50 (56.6) %, positive cultures.

Massachusetts.—Three human outbreaks (one due to minced chicken, one due to locally prepared blood sausage, at Lowell, one cause unknown).

Mr. J. Raymond Sanborn, Department of Microbiology, Massachusetts Agricultural College, Amherst, collected and forwarded, in April, 1921, 5 samples of soil (clover field, poultry yard, cow corral, pasture land and corn field) and 12 specimens of vegetables and feeds: parsnips, cabbage stubs, moldy hay (2), corn stalks (2), carrots, dandelion, lettuce, beans, soja beans and decaying corn stalks. The soil from the pasture land contained B. botulinus, type B, while the parsnips and beans produced weak toxins.

Summary: 5 cultivated and manured soils, 1 type B; 12 vegetables and feeds, 2 weak toxins.

Total: 17 samples with 1 (3), or 5.8 (17.0) %, positive cultures.

Michigan.—Three human outbreaks due to olives and spinach commercially packed in California.

Mr. Frank Gerber, of the Fremont Canning Company, collected, in November, 1920, and forwarded through Mr. A. B. Todd, Director of Inspection for Michigan, National Canners Association, 24 samples of soils (6 specimens each from pea fields, corn fields, Michigan bean fields, and cabbage fields) and 24 specimens of pea vines, corn husks and white bean vines and roots.

One sample each of the corn husks, white bean vines, pea vines and soil of corn garden produced weak toxins.

In July, 1921, Mrs. Zae Northrup Wyant, Research Associate in Bacteriology, Michigan Agricultural College, East Lansing, collected and submitted for examination 28 samples of soil: newly cultivated bean field, bean field, oat field, corn garden, pea garden, potato garden, clay soil from garden, soil from garden (2), soil from newly plowed field, soil from under mulch on strawberries, soil from barnyard, soil from vineyard, soil from 7 pens poultry house, pig pen, yard, alfalfa field, pea patch, cucumber patch, barnyard, timothy field, corn field, bean field, potato field, wheat field, garden soil, pole bean garden, poultry ranch, 5 specimens of silage (from bottom of Bacteriology barn silo); 5 samples of feeds (lettuce, new timothy and clover hay, dried beet pulp, ear corn, corn flakes) and 14 samples of manure and compost. The following cultures were positive:

Silage northwest silo, Dairy barn, 2 feet deep only; B. botulinus, type B (48 hour toxin).

Soil from underneath mulch on strawberries, College Farm, B. botulinus, type B.

Soil and bedding from pig pen and yard, west end of barn, weak toxin.

Chicken manure (Wyant); B. botulinus, type A.

In September, 1921, Dr. J. C. Geiger obtained 10 samples of virgin soil from wooded areas (20 to 30 miles from Detroit) and 10 samples of garden soil from the Tuberculosis Farm near Detroit. Two cultures prepared with garden soil contained B. botulinus, type B, and 2 other cultures B. tetani.

Summary: 82 fertilized or manured soils, 3 type B, 2 weak toxins, 2 B. tetani; 10 virgin soil, all negative; 5 silage, 1 type B; 29 vegetables and feeds, etc., 3 weak toxins; 14 manure and compost, 1 type A.

Total: 120 samples with 5 (10), or 4.1 (8.3) %, positive cultures.

Minnesota.—The Minnesota Valley Canning Company, Le Suer, submitted in December, 1920, 4 samples of soil (corn ground [2] and pea fields [2]) and 4 specimens of corn husks, pea plants and roots. The cultures prepared with these samples were all negative. Additional samples of soil (4) and plant products (4) were sent from other farms of the same company. The cultures were nontoxic.

Mr. C. D. Geidel, Director of the Minnesota Inspection Service, National Canners Association, St. Paul, forwarded in January, 1921, 6 samples of soil (3 different farms) and 4 specimens of corn roots and corn leaves collected by the Barr Pickling and Preserving Company of St. Cloud. One culture of the upland soil, Barr Farm, contained B. botulinus, type A, while 2 other soil specimens produced weak toxins.

In October, 1921, Dr. J. C. Geiger collected 10 samples of virgin soil from Minnehaha Creek in the vicinity of Minneapolis and 10 samples of soils from vegetable gardens. One culture of garden soil contained B. botulinus, type A and one specimen of virgin soil produced a weak toxin. Two garden soils contained B. tetani.

Summary: 24 cultivated or manured soils; 2 type A and 2 weak toxins; 10 virgin soil, 1 weak toxin; 12 roots, leaves, vegetables, etc., all negative.

Total: 46 samples with 2 (5), or 4.3 (10.8) %, positive cultures.

Mississippi.—Dr. Charles F. Briscoe, Bacteriologist, Mississippi Agricultural and Mechanical College, Agricultural College, collected and forwarded, in May, 1921, 6 samples of soil (cow stable, pig pen, poultry pen, clover field, unknown [2]) and 13 specimens of vegetables, feeds, roots, etc.: decaying vegetation (2), bean pods and stalks, moldy hay (2), pea pods and stalks, corn stalks, turnip roots, radish tops, beet roots, carrot roots, fresh lettuce and beet tops. The following cultures were toxic:

Decaying vegetation, B. botulinus, type B.

Corn stalks, B. botulinus, type B.

Turnip roots, B. botulinus, type B.

One sample each of soil, moldy hay and carrot roots gave weakly toxic cultures.

Summary: 6 cultivated or manured soils, 1 weak toxin; 13 vegetables, feeds, etc., 3 B. botulinus, type B, 2 weak toxins.

Total: 19 samples with 3 (6), or 15.7 (31.5) %, positive cultures.

Missouri. — Prof. William A. Albrecht, Department of Soils, College of Agriculture, University of Missouri, Columbia, collected and forwarded, in March, 1921, 7 samples of continuously cropped and manured soil (blue grass

pasture, hog lot, rye field, cattle lot, chicken yard, corn lot, clover field) and 7 specimens of vegetables, compost, etc. (ensilage [2], corn stalks, decaying blue grass hay, leaf compost, blue grass manure, fresh lettuce) and jars of spoiled home canned beans. The following cultures were toxic:

Soils from blue grass pasture, hog lot and corn field, 3 B. botulinus, type B.

Ensilage fresh collection, 1 B. botulinus, type B.

Decaying blue grass hay, 1 B. botulinus, type B.

Manure blue grass, 1 B. botulinus, type B.

Home canned beans (nontoxic brine), 1 B. botulinus, type A.

One of us (B. J. D.) collected, in July, 1921, near the Campus of the University of Missouri, 10 samples of soil (street, driveway, clay soil, top soil, turned up soil near pond, walk near dairy), wheat (2), and manure (2), and 7 specimens of vegetables, feeds and manure (decomposed hay [2], wheat [3], manure [2]). The following cultures were toxic:

Soils, near pond, near dairy, wheat field (2), 4 B. botulinus, type B.

Two soil samples and one specimen of wheat gave weak toxin.

In June, 1921, the laboratory received from Peruque, two small wild ducks. The cadavers were badly decomposed, but coccidia were found in the liver and intestines. Cultures were taken from a composite sample of the intestinal tract and B. botulinus, type B was demonstrated in these specimens.

Summary: 17 soils continuously cropped and manured, etc., 7 type B and 2 weak toxins; 14 vegetables, ensilage, manure, 3 type B and 1 weak toxin; 1 intestines of wild ducks, 1 type B; 2 home canned string beans, 1 type A.

Total: 34 samples with 12 (15), or 35.2 (44.1) %, positive cultures.

Montana.—One human outbreak at Java, due to stuffed ripe olives grown in California.

Dr. D. B. Swingle, Department of Botany and Bacteriology, Agricultural Experiment Station, University of Montana, Bozeman, collected and forwarded, in September, 1921, 9 samples of soil and dirt (rabbit yard, clover field, corn field, chicken pen, pig corral, sheep pen, horse pen, grain field, duck pen) and 16 specimens of vegetables, manure, silage, etc. (carrots, moldy alfalfa, beans, decayed beets, rutabaga, peas, summer squash, lettuce, corn silage, tomatoes, crab apple, cabbage leaves, parsley, spinach and cucumbers, cow manure). The following cultures were toxic:

Carrots, lettuce, corn silage, 3 type A.

Moldy alfalfa, 1 type A and B.

Dirt, horse pen, 1 type B.

Parsley, green tomatoes, rutabaga and summer squash, 4 weak toxins.

Dr. J. C. Geiger collected, in September, 1921, 41 samples of soil from 3 different areas in Glacier National Park. The cultures gave the following results:

Area 1: West side of Continental Divide along Lake Ellen Wilson and Gunsight Pass—12 samples, 5 type A.

Area 2: East side of Continental Divide, foot of Swift Current Pass and Mt. Wilbur—16 samples, 2 type A and 2 type B, 2 weak toxins.

Area 3: Foothills of eastern side of Continental Divide (Midvale Creek and Squaw Mountain)—13 samples, 2 type A and 5 weak toxins.

Summary: 9 soils or dirt from manured places, 1 type B; 41 virgin mountain soils, 9 type A, 2 type B; 7 weak toxins; 16 vegetables, feeds, etc., 3 type A, 1 type A and B, 4 weak toxins.

Total: 66 samples with 16 (27), or 24.2 (40.9) %, positive cultures.

Nebraska.—Dr. L. Van Es, Department of Animal Pathology and Hygiene, University of Nebraska, Lincoln, forwarded, in May, 1921, 12 samples of soil (poultry yard, cow yard, sheep yard, cattle yard, hog yard, wheat field, corn field, field No. 8, small bull pasture, hog yard (serum plant), horse yard, alfalfa field) and 11 specimens of vegetables, feeds, etc. (bottom of old hay stacks [2], corn stalks, corn silage, lettuce, alfalfa, decayed cabbage stalks, old manure pile [2], dandelions, old beets from root cellar). The following cultures were toxic:

Old manure pile (Havelock), 1 B. botulinus, type A.

Dandelions, 1 B. botulinus, type A.

Soil, poultry yard, hog yard, wheat field, alfalfa field, gave weak toxins.

Summary: 12 cultivated or manured soils, 4 weak toxins; 11 vegetables and manure pile, 2 type A.

Total: 23 samples with 2 (6), or 8.6 (26.0) % positive cultures.

Nevada.—Five chicken outbreaks, due to home canned string beans (1), moldy potatoes (2), spoiled sour milk (1) and spoiled canned goods (1).

Dr. J. C. Geiger brought, in December, 1920, 3 samples of field soil and of garden soil from the vicinity of Reno and 2 samples of garden and 2 of desert soil from Hastings. One sample of garden and one of desert soil produced weakly toxic cultures. The remainder was nontoxic.

Mr. G. D. Delprat, Jr., collected in Sage County near the California border 3 samples of virgin soil. Two contained B. botulinus, type A.

Dr. L. R. Vawter, University of Nevada, forwarded, in December, 1921, 11 samples of soil. The cultures gave the following results:

Virgin soil along road through secret pass in the Ruby Mountains (7,000 feet)—1 type A (south of Elko).

Sage brush bench, north of Elko, used as a range for cattle and sheep—1 type A.

Creek bed soil, Gardnerville—1 type A.

Soil alfalfa field, Truckee Valley, irrigated 8 miles south of Reno—1 type A.

Virgin sage brush soil at foot of Mt. Rose (20 miles southwest of Reno)—1 type A.

Lake bed soil, bank of Little Washoe Lake, in Washoe Valley—1 type A.

Garden soil in Reno (avian botulism), hayfield, and river bank adjoining Humboldt River, Elko, garden soil in Gardnerville and Steamboat Springs, Geyser Basin—all negative.

Summary: 5 field soil, 1 B. botulinus, type A; 8 garden soil, 1 B. botulinus, type A, 1 weak toxin; 4 desert and sage brush, 2 B. botulinus, type A, 1 weak toxin; 8 virgin soil, 5 B. botulinus, type A.

Total: 25 samples with 9 (11), or 36.0 (44.0) %, positive cultures.

Several isolations of B. botulinus, type A and B from the livers of cattle by Dr. L. R. Vawter should be added.

New Hampshire.—Prof. Mabel Brown, New Hampshire Agricultural Experiment Station, Durham, collected and forwarded 6 samples of soil (cattle corral, poultry yard, oat field, corn field, pig pen, clover field) and 8 specimens of vegetables and feeds (radishes, bean stalk, pea stalk, beets, decaying leaves, lettuce, hay and pea pods).

The sample of hay contained B. botulinus, type B; the pea stalk gave a weak toxin.

Summary: 6 cultivated or manured soils, all negative; 8 vegetables and feeds, 1 B. botulinus, type B, 1 weak toxin.

Total: 14 samples with 1 (2), or 7.1 (14.2) %, positive cultures.

New Jersey.—One human outbreak, due to home canned string beans, at Newark.

Dr. J. H. McNeil, Chief, Bureau of Animal Industry, Department of Agriculture, Trenton, forwarded, in February, 1921, 5 samples of soil collected from salt meadows near Toms River and Osbornville, eastern section of the state. One sample from J. Farm, one from Westervelt and one from Johns Farm contained B. botulinus, type B.

Dr. Fred Boerner, Jr., Bureau of Animal Industry, Pennsylvania Department of Agriculture, Philadelphia, collected and forwarded, in February, 1921, 20 samples of soil and feeds from 8 different localities. The following results were obtained with the cultures:

Soil and sod from salt meadow, Cape May, 2 B. botulinus, type B.

Soil, mixture of hay (1), hay, Swainton, 1 weak toxin.

Soil and corn, South Dennis, all negative.

Soil (2), forage alfalfa, corn, woodbine, all negative (4).

Soil, corn, salt hay, corn stover, near Goshen, all negative.

Soil, Dias Creek, negative (1).

Soil, feed mixture, corn stover, salt hay, all negative (4).

Soil, Rio Grande, 1 weak toxin.

Two samples of soil collected from tomato field near Camden were cultivated, in February, 1921, and found to be free from B. botulinus spores.

Dr. F. G. Steinbach of Wildwood submitted, in October, 1921, 3 samples of soil (H. and F. Farms) one of manure (1), and 4 of feeds (alfalfa, moldy bread, corn stubble and stover). The cultures gave the following results:

Soil H. farm, 1 B. botulinus, type A.

Soil F. farm, 1 B. botulinus, type B.

Summary: 20 meadow soils and sod from salt farms, 6 type B, 1 type A, 2 weak toxins; 1 manure, negative; 15 feeds, hay corn, etc., all negative.

Total: 36 samples with 7 (9), or 19.4 (25) %, positive cultures.

New Mexico.—Mr. Harold Gray, State Engineer, Santa Fe, forwarded, in December, 1920, 8 samples of soil supposedly collected in various sections of the state. The cultures prepared from these samples were all nontoxic.

New York.—Five human outbreaks, one in New York City, due to ripe olives grown and packed in California, one to home prepared cottage cheese in the western part of the state, one to home canned corn, one to commercially packed spinach and one to unknown food products, the last two in New York City.

S. K. Farrar, Assistant Director of Inspection, National Canners Association, Rochester, forwarded, in January, 1921, 3 samples of soil (Red Creek and Oswego) and 4 specimens of cabbage leaves, corn husks and pea vines. The cultures gave the following results:

Soil, Oswego, B. botulinus, type A.

Cabbage from field near Red Creek, B. botulinus, type A.

Peavine, weak toxin.

Dr. George A. Stock, Medical Examiner, U. S. Veterans Bureau, forwarded, in October, 1921, 24 samples of virgin soil collected from Mount Baker, which is in the vicinity of Saranac Lake. Four samples contained B. botulinus, type A, 1 gave a weak toxin and 3 contained B. tetani.

Summary: 3 cultivated and manured fields, 1 type A; 24 virgin soils, 4 type A, 1 weak toxin; 4 vegetables, etc., 1 type A, 1 weak toxin.

Total: 31 samples with 6 (8), or 19.3 (25.8) %, positive cultures.

North Carolina.—Dr. F. A. Wolf, Department of Botany, North Carolina Agricultural Experiment Station, West Raleigh, collected and forwarded, in

April, 1921, 12 samples of soil (cow pen, vineyard, garden, cattle and sheep pasture, oat field, pig pen, apple orchard and chicken pen, clover field and corn field, sheep pen and horse pen) and 8 specimens of vegetables and feeds (cabbages, peas, lettuce, chard, onions, corn stalks, beans and radishes). The following cultures contained weak toxins, which could not be identified by toxin-antitoxin tests: soil from vineyard, garden, cattle pasture, radishes, chard and onions.

Summary: 12 cultivated soils, 3 weak toxins; 8 vegetables, 3 weak toxins.

Total: 20 samples with 6, or 30%, toxic cultures.

North Dakota.—Dr. A. F. Schalk, North Dakota Agricultural College, collected and forwarded, in May, 1921, 7 samples of soil (cattle corral, poultry yard, corn field, clover field, pig pen, pasture, grain field) and 7 specimens of vegetables and feeds (radishes, moldy straw, forage, corn stalks, decaying roots [2] and lettuce).

The culture prepared with the forage contained B. botulinus, type A, while those made from the moldy straw and corn stalks were weakly toxic.

Summary: 7 cultivated soils, all negative; 7 vegetables and feeds, 1 type A, and 2 weak toxins.

Total: 14 samples with 1 (3), or 7.1 (21.4) %, positive cultures.

Ohio.—Two human outbreaks, at Canton, one due to ripe olives packed in California and one at Canaan, due to spinach.

The Sears Nichols Canning Company at Chillicothe submitted, in June, 1920, 2 samples of beets and soil. Six cultures were prepared and found to be nontoxic. In October, 1920, the same company forwarded 5 samples of soil (beet field [2], lima bean field, sweet corn field and spinach field) and 7 specimens of beets, lima beans, green corn and raw spinach. A sample of soil from the sweet corn field contained B. botulinus, type B.

Mr. A. M. Wadsworth, Director of Inspection, National Canners Association, Columbus, forwarded, in December, 1920, from Canal Winchester, 6 samples of soil and 8 specimens of vegetables and plant remnants from pea, lima bean and corn fields.

One sample of lima beans from G. B. farm contained B. botulinus, type B.

One sample of corn husks from C. A. farm produced a weak toxin.

M. A. Bates, Assistant Director of Inspection, National Canners Association, Columbus, forwarded, in December, 1920, 5 samples of soil and 8 specimens of vegetables and plant remnants from a cabbage field near Fremont and Bellevue, corn and strawberry fields near Elyria, tomato fields near Madison and corn fields near DeGraff. A culture prepared with cabbage leaves from Bellevue contained B. botulinus, type B and another from Fremont produced a weak toxin.

Summary: 18 cultivated soils, 1 type B; 23 vegetables or plant remnants, 2 type B and 2 weak toxins.

Total: 41 samples with 3 (5), or 7.3 (12.1) %, positive cultures.

Oklahoma.—Dr. L. L. Lewis, Oklahoma Agricultural and Mechanical College, Stillwater, forwarded, in April, 1921, 10 samples of soil (one-half to 5 inches depth, barnyard, poultry, sheep, cattle yard, hog lot) and 14 specimens of vegetables and plant remnants (decaying canna tops, decaying cauliflower, cinari, spinach tops, lettuce, tomatoes, sweet potatoes, radish roots, horse radish, onion roots, pea tops, decayed wheat straw, salsify roots and decayed cane hay). One culture prepared from decaying canna tops contained B. botulinus, type A. Horse radish, decayed cane straw and pea tops gave weak toxins.

Summary: 10 cultivated or manured soil, negative; 14 vegetables and plant remnants, 1 type B, 3 weak toxins.

Total: 24 samples with 1 (3), or 4.1 (12.5) %, positive cultures.

Oregon.—Four human and 2 fowl outbreaks; one at Hillsboro, due to home canned corn, one at Ontario, one at Klamath Falls and one at Grants Pass, all due to home canned string beans; 2 fowl outbreaks were caused by home canned string beans at Klamath Falls.

In August, 1920, E. Wagner collected and brought to the laboratory from Ashland, 3 samples of soil (barnyard, strawberry bed, pea garden); 2 specimens of fruits from the trees (cherries, bird pecked and sound) and 7 specimens of vegetables (beets, peas, and sugar peas, asparagus, bean vines, moldy vines, leaf mold, strawberries). The following results were obtained:

Sugar peas, leaf mold and asparagus tips contained B. botulinus, type A.

Soil from pea garden, barnyard and asparagus, beets, pea vines, moldy leaves (2), bean vine and cherries gave 9 weakly toxic cultures.

Dr. J. C. Geiger collected, in February, 1920, at Klamath Falls 2 samples of soil and 3 specimens of bean stalks and beans (chicken outbreak 26). The soil, one bean and the bean stalks contained B. botulinus, type A.

Dr. A. A. Soule of Klamath Falls forwarded, in September, 1921, 15 samples of soils. The cultures prepared with these specimens gave the following results:

Virgin soil (2), 2 B. botulinus, type A.

Garden soil (3), city pest house (1), 4 B. botulinus, type A.

Surface soil and swampy soil (2), weak toxins.

Garden soil (1), road soil (1), tule soil (1), tannery slough soil (1), surface dirt (1), spring yard (2), all negative.

Dr. H. J. Sears, Department of Bacteriology, University of Oregon Medical School, Portland, collected 2 samples of virgin, 3 of cultivated and 1 of nonfertilized soil (chicken yard) from the vicinity of Markham Hill. The cultures prepared with these specimens were nontoxic.

Summary: 4 virgin soils, 2 type A; 19 garden soils, barnyards, swamp soil, etc., 6 type A and 4 weak toxins; 12 fruits and vegetables, 5 type A and 7 weak toxins.

Total: 38 samples with 13 (24), or 34.2 (57.8) %, positive cultures.

Pennsylvania.—Two human outbreaks, one at Pittsburgh, due to home canned corn and one at Greensburg, caused by pickled olives packed in California.

Dr. George H. Hart, University of Pennsylvania Extension School, Philadelphia, forwarded, in April, 1921, 6 samples of soil (corral, corn field, poultry yard, pig pen, pasture, rye field) and 2 specimens of corn husks and beets. The following cultural results were obtained:

Soil from corn and rye field, 2 B. botulinus, type B.

Soil from field, pasture for 50 years, 1 B. botulinus, type A.

Soil from corral, 1 weak toxin.

In July, 1921, an additional 6 samples of moldy hay and corn from Montgomery and Chester counties, as well as moldy silage and hay were sent by Dr. Hart. Two specimens of silage were received from Pittsburgh, in May, 1921. The following cultures were toxic:

Sample of moldy hay, Chester County, B. botulinus, type B.

Sample of moldy corn fodder, B. botulinus, type A.

Summary: 6 manured or cultivated soils, 2 type B, 1 type A, 1 weak toxin; 10 feeds, silage, moldy hay, 1 type B, 1 type A.

Total: 16 samples with 5 (6), or 35.0 (37.5) %, positive cultures.

Rhode Island.—Dr. H. G. May, Agricultural Experiment Station of Rhode Island State College, Kingston, forwarded, in August, 1921, 6 samples of soil (rabbit pen, chicken yard, grain field, corn field, cattle pen and pasture) and 18 specimens of vegetables and feeds: silage, turnips (2), straw (2), dried beans, hay, beans (2), clover, beets (2), lettuce (2), spinach, peas, carrots and beet tops. The cultures prepared with these specimens proved nontoxic. One culture of spinach and soil of a corn field contained B. tetani.

Summary: 6 soils (cultivated or manured), all negative; 18 vegetables and feeds, all negative.

Total: 24 samples free from spores of B. botulinus.

South Carolina.—Dr. W. B. Aull, Associate Professor of Bacteriology, the Clemson Agricultural College, Clemson College, forwarded, in August, 1921, 7 samples of soil (hog pen, corn field, cow corral, grain field, poultry yard, clover field, cow pasture) and 15 specimens of vegetables and feeds (decayed leaves [2], beet tops, carrots [2], bean pods and stalks, beets, tops of kale, moldy hay [2], lettuce, pieces of corn stalks, tops of turnips [3]). The following cultures were toxic:

Beet and carrot tops, bean pods and stalks, 3 B. botulinus, type B.

Soil from grain field, 1 B. botulinus, type B.

Turnips, 1 weak toxin.

Summary: 7 soils manured or cultivated, 1 type B; 15 vegetables and feeds, 3 type B, 1 weak toxin.

Total: 22 samples with 4 (5), or 18.1 (22.7) %, positive cultures.

South Dakota.—Mr. C. D. Geidel, Director of the Minnesota Inspection Service, National Canners Association, St. Paul, forwarded, in January, 1921, 2 samples of soil and 2 specimens of corn husks procured from the Bigstone City Canning Company, Bigstone City. The cultures prepared with these specimens were nontoxic.

Dr. T. B. Taylor, Animal Health Laboratory, South Dakota State College, Brookings, forwarded, in April, 1921, 7 samples of soil (corn field [2], clover field, pig pen, cattle corral, pasture and poultry yard) and 6 specimens of roots (2), corn stalks, moldy hay and decaying vegetables (2). The cultures prepared with these were all nontoxic.

Summary: 9 soils cultivated or manured, negative; 18 vegetables and feeds, negative.

Total: 27 samples entirely free from spores of B. botulinus.

Tennessee.—One human outbreak in Memphis, due to ripe olives packed in California.

Dr. Maurice Mulvania, Department of Bacteriology, University of Tennessee, Knoxville, forwarded, in May, 1921, 7 samples of soil (corn field, cattle pasture, grain field, clover field, cattle pen, hog pen, poultry yard) and 13 specimens of vegetables and feeds (corn stalks, bean stover, bean stalk, pea vines, parsley, mustard, Irish potatoes, lettuce, beet stalk, young cabbage plant, straw [3]). The following cultures were toxic:

Irish potatoes, bean stalks, beet stalks, 3 B. botulinus, type A.

Soil from clover field, lettuce, mustard and decayed vegetation, 3 weak toxins.

Summary: 7 soils, cultivated or manured, 1 weak toxin; 13 vegetables and feeds, 3 type A, 2 weak toxins.

Total: 20 samples with 3 (6), or 15.0 (30.0) %, positive cultures.

Texas.—One human outbreak, due to sausages prepared in El Paso.

Mr. E. B. Reynolds, Division of Agronomy, Texas Agricultural Experiment Station, College Station, forwarded, in May, 1921, 7 samples of soil (cattle

corral, pig pen, poultry yard, corn, grain, clover and pasture fields) and 14 specimens of vegetables and feeds (corn stalks, decaying vegetation [2], moldy hay [2], bean pods and stalks, peas, lettuce, beet tops, turnip greens [2], beets, radishes and parsley). The cultures were all nontoxic.

Summary: 7 soils, cultivated or manured, all negative; 14 vegetables and feeds, all negative.

Total: 21 samples entirely free from spores of B. botulinus.

Utah.—Dr. T. B. Beatty, State Health Commissioner, Utah State Board of Health, Salt Lake City, forwarded, in December, 1920, 2 samples of garden soil and 2 of unbroken sage brush, surface soil from the vicinity of Salt Lake City. One sample of garden soil contained B. botulinus, type A.

Vermont.—Professor B. F. Lutman, Department of Botany, University of Vermont, Burlington, collected and forwarded, in May, 1921, 9 samples of soil (pasture land, pig pen, cattle yard, corn field, chicken pen, forest humus, clover field, pig yard, barley field) and 13 specimens of vegetables, feeds, etc. (chaff [2], moldy silage, forest leaves, corn stalks, radishes, decaying weeds from compost heap, straw, bean pods, potatoes, beets, bean stalks and droppings from hen roost). The following cultures were toxic:

Decaying weeds from compost heap, 1 B. botulinus, type B.

Soil from pig yard, 1 B. botulinus, type B.

Summary: 9 soils cultivated or manured, type B; 13 vegetables, feeds and decaying vegetation, type B.

Total: 22 samples with 2, or 9.0%, positive cultures.

Washington.—Fourteen human and 23 fowl outbreaks; one human outbreak each at Seattle, due to home canned string beans and asparagus; one at McKenna, due to home canned spinach, 6 at Yakima, due to home canned corn, one at Yakima, due to home canned asparagus, one at Toppenish, due to home canned spinach, one at Yakima, due to commercially packed milk, one at Walla Walla, due to home canned asparagus and one at Monroe, due to commercially packed ripe olives. One chicken outbreak at Yelm, due to home canned string beans, 4 at Yakima, due to home canned corn and 2 due to home canned peas and 16 due to buried raw potatoes.

Mr. A. W. Hansen, Chief, Seattle Station, Bureau of Chemistry, U. S. Department of Agriculture, Seattle, forwarded, in January, 1921, 6 samples of virgin soil taken at Roosevelt Heights, just north of Seattle, 10 samples of commercial truck garden soil from South Park, sample of manure and 4 specimens of vegetables (potatoes, onions, carrots and turnips). The cultures prepared with these specimens were all nontoxic.

Dr. C. R. Fellers, Pacific Fisheries Investigation, National Canners Association, Seattle, collected and forwarded, in February, 1921, from the vicinity of the University grounds 11 samples of soil (garden, lumber yard, beet garden, subsoil, mud and silt, sewer opening, virgin, marsh soil, flower beds, drug plant garden [2]) and 1 sample of manure. The specimen of mud and silt produced a weakly toxic culture.

Dr. J. C. Geiger collected, in May, 1921, near Toppenish, Yakima Valley, 34 samples of garden soil (spinach garden [10], garden [18], irrigation ditches [4] and field soil [2]). The cultures gave the following results:

Spinach garden, 1 B. botulinus, type A, 2 type B, 1 weak toxin.

Vegetable gardens, 3 B. botulinus, type A.

Similar samples were collected at Tieton, Yakima Valley: 10 samples of ranch soil and 4 specimens of soil and potatoes from pits suspected of con-

taining feed which caused avian botulism. The specimens developed toxic cultures, as follows:

Ranch soil, 3 B. botulinus, type A, 2 type B, 1 weak toxin.

Potato pits, 2 B. botulinus type A, 1 type B.

Additional samples were collected at random in the Yakima Valley: 6 samples of virgin soil, 6 of garden soil and 3 of pea garden soil. The following specimens developed toxic cultures:

Virgin soil, 5 B. botulinus, type A.

Garden soil, 1 B. botulinus, type A, 1 weak toxin.

Dr. J. C. Geiger collected, in July, 1921, near Yelm, 37 specimens of soil, manure, home canned peas and beans, etc. The following specimens furnished toxic cultures:

Eighteen soils, hen house (1), bean garden (6), cultivated (5), wheat field (3), pasture (1), garden (2), 17 B. botulinus, type A; 4 soil from irrigation ditches, 4 B. botulinus, type A; 3 virgin soils, 2 B. botulinus, type A, 1 weak toxin; 6 manures (hogs 3, cattle 3), 3 B. botulinus, type A, 1 weak toxin; 3 soil from house excavation, 3 B. botulinus, type A; 3 home canned peas (2), beans (1), 1 peas, 1 B. botulinus, type A.

In September, 1921, Dr. J. C. Geiger collected 20 samples of virgin soil along the eastern ledge of the Nisqually Glacier, Paradise Park, of the Mount Rainier National Park (elevation 5,000 to 6,000 feet); 7 cultures contained B. botulinus, type A, one type B and 5 gave weak toxins.

Summary: 36 virgin soils, 14 type A, 1 type B, 6 weak toxins; 41.6 or (58.3) %, positive; 98 garden, or irrigated soils, 32 type A, 4 type B, 4 weak toxins; 36.7 (40.8) % positive; 8 manure, 3 type A, 2 weak toxins; 8 vegetables and potatoes, 2 type A, 1 type B; 3 home canned peas or beans, 1 type A.

Total: 153 samples with 58 (70), or 37.8 (45.7) %, positive cultures.

West Virginia. — One human outbreak at Parkersburg due to pumpkin packed in Ohio.

Mr. Francis M. Morgan, Department of Soils, College of Agriculture and Agricultural Experiment Station, West Virginia University, Morgantown, collected and forwarded, in April, 1921, 8 samples of soils (poultry yard, cattle yard, horse pasture, clover, corn and wheat fields, pig pen, cold frames green house) and 12 specimens of moldy straw, corn stalks, decayed oat straw, cabbage heads, green peas, green beans, humus from compost heap, carrots, cucumber, radishes and manures from pig pen and horse stable.

One sample of soil from a field used for corn last year contained B. botulinus, type B.

Dr. J. C. Geiger collected 21 samples of virgin soil from the Alleghany Mountains in West Virginia.

One sample contained B. botulinus, type B, and one B. tetani.

Summary: 21 virgin soils, 1 type B; 8 manured and fertilized soils, 1 type B; 12 vegetables and manure, negative.

Total: 41 samples with 2, or 4.8%, positive cultures.

Wisconsin.—One human outbreak, due to commercially prepared sausages, at Milwaukee.

Mr. H. C. Kitchen, Director of Wisconsin Inspection Service, National Canners Association, Madison, forwarded, in December, 1920, 2 samples of soil and corn husk each from Columbus, 1 sample of pea vines and soil each, Cedar Grove, Thiensville and Bloomer. Ten cultures were prepared. One soil sample from Cedar Grove, Sheboygan County, produced a weakly toxic culture.

Dr. J. C. Geiger collected, in October, 1921, 19 samples of virgin soil with pine decay from wooded land, vicinity of Milwaukee, and 9 samples from a celery farm of the same region.

One sample of soil from wooded, virgin soil contained B. botulinus, type B, and one other produced a weak toxin.

One sample of soil collected from a celery farm gave a weak toxin, while three others contained B. tetani.

Summary: 14 cultivated and manured soils, 1 weak toxin; 17 virgin soil, 1 type B, 1 weak toxin; 5 vegetable remnants, negative.

Total: 36 samples with 1 (3), or 2.7 (8.3) %, positive cultures.

Wyoming.—One human outbreak at Cheyenne, due to home canned corn.

Dr. Cecil Elder, College of Agriculture and Mechanical Arts, University of Wyoming, Laramie, collected and forwarded, in April, 1921, 12 samples of soil [cattle corrals (6), pasture (2), hog pen (1), poultry yard, grain field and clover field] and 10 specimens of feeds and vegetables [moldy hay (5), moldy barley, pea pods and stalks, parsnips, carrots and potato].

The specimen of moldy barley contained B. botulinus, type A. The soil samples from the poultry yard, cattle corral, Ranch (1) and potatoes produced weak toxins.

In June, 1921, one of the writers (K. F. M.) collected 30 soil samples in the Yellowstone National Park. The cultures prepared gave the following results:

Forest soil near Old Faithful (2), 1 type A; upper Geyser Basin (9), rock-slide near Butterfly Geyser (1), all negative; road embankment near Castle Geyser (1), 1 type A; Forest soil near Saw Mill Geyser (2), 1 type A; Blacksand Basin (forest 2, swampy meadow 1), all negative.

Vicinity of Yellowstone Lake (8): Lake sand, near Hotel (1), negative; soil of damp meadow near Camp (1), negative; fresh soil cut near road side, Camp (1), 1 type A; soil on tree roots (2), all negative; soil from horse corral (1), 1 type A; soil from Stevenson Island (2), 2 type A.

Grand Canyon of the Yellowstone (8): Red volcanic rock near Lower Falls (1), street cut near Lookout Point (1), Terrace near Lower Falls (1), Meadow near hotel (1), sidewalk Lower Falls (1), all negative; damp soil under glacial bowlder near Canyon (1), 1 type B; summit of Mt. Washburn, damp soil (2), 2 type A.

Mammoth Hot Springs (5): Soil, squirrel hole on meadow (1), forest soil near Angel Trail (1), Devil's Kitchen (1), all negative; garden soil near hotel (1), 1 type A; soil near Boiling River (1), 1 type B.

Summary: 28 virgin soil (Yellowstone Park), 8 type A, 2 type B; 14 cultivated manured or garden soil, 2 type A, 2 weak toxins; 10 vegetables and feeds, 1 type A, 1 weak toxin.

Total: 52 samples with 13 (15), or 25.0 (38.8) %, positive cultures.

The statistical data presented in the preceding pages are summarized in table 1.

A brief analysis shows that 1,538 specimens, exclusive of California, have been studied; 375, or 24.3%, of the samples produced toxic cultures, although only 256, or 16.5%, could be identified by an antitoxin neutralization test. These figures are slightly changed by adding the findings reported for California, namely, 26.8%, or about one-quarter, of the field specimens collected in the United States contained spores,

which produced a B. botulinus-like poison in mass cultures. In 18.2% of the cultures, the presence of B. botulinus was definitely established by antitoxin tests.

TABLE 1

RESULTS OF EXPERIMENTS WITH SPECIMENS FROM VARIOUS STATES

State	Total Number of Specimens Examined	Total Number of Toxic Cultures	Total Number of Typed Cultures	Type A	Type B	Types A and B	Untyped	Percentage Total Toxic Cultures	Percentage Typed Cultures
Alabama	16	1	1	1	—	—	—	6.2	6.2
Arizona	1	—	—	—	—	—	—	0	0
Arkansas	41	6	1	—	1	—	5	14.6	2.4
(California)	(624)	(206)	(139)	(100)	(35)	(4)	(67)	33.0	22.2
Colorado	69	17	12	12	—	—	5	24.6	17.4
Connecticut	24	6	1	1	—	—	5	25.0	4.1
Delaware	18	6	4	—	4	—	2	33.3	22.2
Florida	45	9	8	1	7	—	1	20.0	17.7
Georgia	20	5	5	—	5	—	—	25.0	25.0
Idaho	33	7	4	3	1	—	3	21.2	12.1
Illinois	39	5	2	—	2	—	3	12.8	5.1
Indiana	28	8	5	—	5	—	3	28.5	17.8
Iowa	34	3	1	1	—	—	2	8.6	2.9
Kansas	30	2	2	2	—	—	—	6.6	6.6
Kentucky	17	12	12	1	11	—	—	70.5	(70.5)
Louisiana	48	6	2	2	—	—	4	12.5	4.1
Maine	41	10	10	8	2	—	—	24.3	24.3
Maryland	30	17	15	1	12	2	2	56.6	50.0
Massachusetts	17	3	1	—	1	—	2	17.6	5.8
Michigan	120	10	5	1	4	—	5	8.3	4.1
Minnesota	46	5	2	2	—	—	3	10.8	4.3
Mississippi	19	6	3	—	3	—	3	31.5	15.7
Missouri	34	15	12	1	11	—	3	44.1	35.2
Montana	66	27	16	12	3	1	11	40.8	24.2
Nebraska	23	6	2	2	—	—	4	26.0	8.6
Nevada	25	11	9	9	—	—	2	44.0	36.0
New Hampshire	14	2	1	—	1	—	1	14.2	7.1
New Jersey	36	9	7	1	6	—	2	40.0	19.4
New Mexico	8	—	—	—	—	—	—	0	0
New York	31	8	6	6	—	—	2	25.8	19.3
North Carolina	20	6	—	—	—	—	6	30.0	0
North Dakota	14	3	1	1	—	—	2	21.4	7.1
Ohio	41	5	3	—	3	—	2	12.1	7.3
Oklahoma	24	4	1	—	1	—	3	16.6	4.1
Oregon	38	24	13	13	—	—	11	63.1	34.2
Pennsylvania	16	6	5	2	3	—	1	37.5	35.0
Rhode Island	24	—	—	—	—	—	—	0	0
South Carolina	22	5	4	—	4	—	1	22.7	18.1
South Dakota	17	—	—	—	—	—	—	0	0
Tennessee	20	6	3	3	—	—	3	30.0	15.0
Texas	21	—	—	—	—	—	—	0	0
Utah	4	1	1	1	—	—	—	25.0	25.0
Vermont	22	2	2	—	2	—	—	9.0	9.0
Washington	153	70	58	52	6	—	12	39.2	37.8
West Virginia	41	2	2	—	2	—	—	4.8	4.8
Wisconsin	36	3	1	—	1	—	2	8.3	2.7
Wyoming	52	16	13	11	2	—	3	30.7	25.0
Total (exclusive of California)	1,538	375	256	150	103	3	119	24.3	16.5
Total (inclusive of California)	2,162	581	395	250	138	7	186	26.8	18.2

One hundred and fifty, or 58.5%, of the typed cultures contained B. botulinus, type A; 103, or 40.3%, type B, and 3, or 1.2%, mixture of type A and B. The predominance of type A is probably due to the

findings made in the State of Washington, and is of little significance. In fact, it is not the relative numerical preponderance of one type over the other, but the geographical distribution of the various types which deserves special consideration in subsequent paragraphs. Attention is called to the negative findings made on the samples procured from New Mexico, South Dakota and Texas. It is quite likely that these results indicate an uneven distribution of the spores of B. botulinus, but it would be unwise to conclude from the limited data that the organism is not present in the soil of these states. The specimens collected in Arkansas, Connecticut, Illinois, Indiana, Louisiana, Michigan, Minnesota, Mississippi, North Carolina, North Dakota, Oklahoma, South Carolina, Tennessee and Wisconsin furnished a relatively large percentage of cultures which were weakly toxic and could not be identified by antitoxin tests. It was also noted that the typed cultures prepared with the samples obtained from these states contained only 1 to 5 minimum lethal subcutaneous guinea-pig doses per 2 c c. Previous studies have shown that specimens collected in California, Oregon, Washington, Montana, and other states, yield, as 25 gm. samples during 10 days' incubation in peptic digest broth, toxic cultures which are fatal to guinea-pigs in dilutions of 1 : 1,000 to 1 : 10,000. Not infrequently, B. botulinus has been isolated in a pure state from these cultures, while innumerable attempts made with the specimens from the first named states, in which no strongly toxic cultures were found, have regularly been unsuccessful. Laboratory tests reported in a former paper indicate that a few spores added to soils produce, as a rule, weakly or nontoxic cultures. It is naturally possible that a number of weakly toxic cultures are the result of the recently described anaerobe isolated by Bengtson from the larvae of Lucilia Caesar, although repeated transplants and cultures of new samples of soil which originally gave weakly toxic cultures furnished in a few instances highly potent enrichments containing the toxin of B. botulinus. One is therefore forced to conclude that the field specimens collected in the 14 states mentioned harbored relatively few spores of B. botulinus. The practical significance of this observation will be considered in greater detail in the following.

The data presented in table 1 suggest a number of interesting points, which will be treated under the following headings:

(1) The relative distribution of B. botulinus in different states of the United States.

(2) The distribution of the two types of B. botulinus.

(3) The telluric distribution of the spores.

(4) The relation of the distribution of B. botulinus in the soil and its products to outbreaks of human and animal botulism.

(1) *The Relative Distribution of B. botulinus in Different States of the United States.*—With the exception of Virginia, every state of the Union supplied from 1 to 624 samples of soil and its products. Nevertheless, the survey cannot be considered in any way complete or exhaustive. Such a study is obviously very difficult and would consume an enormous financial outlay and several years of continuous work. A scrutiny of table 1 indicates that a number of states provided a small number of specimens which necessarily represent confined areas of the state. These facts should be fully appreciated and the deductions which will be made from the 1,538 cultural findings should be considered tentative, although they are in many ways suggestive.

For the sake of clearness and brevity, the figures dealing with the percentage of toxic and properly identified cultures are presented on chart 1. Four contrasting shades representing the percentages of 1 to 10 (1), 10 to 20 (2), 20 to 30 (3), and 30 to 70 (4), have been chosen for graphic illustration of the distribution in the different states. A rapid glance at chart 1 reveals three interesting facts, namely:

(1) The spores of B. botulinus are relatively rare in the samples of soil, etc., collected from the Middle States, the Great Plains States and those bordering the Great Lakes and the Mississippi River with its tributaries, the Missouri, Arkansas and Red, Des Moines and Illinois Rivers.

(2) The Atlantic States supplied specimens which produced frequently from 10 to 30% toxic cultures.

(3) The soil of the Pacific Coast and Rocky Mountain States was heavily infected with B. botulinus.

One state of the 14, bordering on the Mississippi River, namely, South Dakota, supplied nontoxic cultures, while Kentucky furnished 70.5%, Mississippi and Tennessee 15% and Missouri 35.2% toxic cultures. In Kentucky and in Missouri, collections were made in the vicinity of experiment stations. As far as Kentucky is concerned, it is known that for a number of years work on animal botulismus has been carried out at the experiment station, and it is not unlikely that the percentage of positive specimens can be attributed to contamination of the premises with spores. The samples from Tennessee originated

in the neighborhood of Knoxville, or in close proximity to the Appalachian Mountains. Excluding these 4 states from consideration, it is clear that the soil of the Mississippi Basin contains the spores of B. botulinus in exceptional instances only. In fact, 9 states which are represented in this study by 311 specimens or one-fifth of all the samples tested in the United States, exclusive of California, gave less than 4.5% positive cultures. Sixty-four per cent. of these toxic cultures were weakly toxic and were probably the result of a very few heat resistant, viable spores.

The states of Michigan, Indiana, Ohio, possibly Pennsylvania and West Virginia, form a part of a region which has a low B. botulinus spore index. This statement is particularly well supported by the

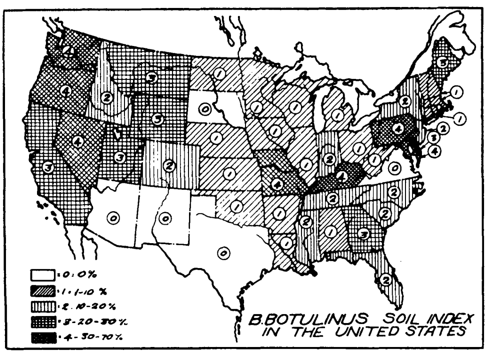

Chart 1

findings made on 120 specimens collected in Michigan; 62 soil samples derived from cultivated fields gave 3, or 4.8%, toxic cultures, while 10 virgin samples proved on repeated tests to be free from B. botulinus. Ohio and West Virginia gave 7.1 and 4.8% toxic cultures. One locality in Indiana supplied 17.8% positive samples, which contained the toxicogenic anaerobe, but judging from the number of additional negative specimens, this state can well be included in the group mentioned. It is regrettable that most of the material on which the data for Pennsylvania are based were collected in the eastern section of the state, and no information relative to the frequency of B. botulinus in the western part along the Alleghany and Ohio Rivers is available. Notwithstanding this fact, it appears that the valley of the Ohio River shares the same low percentage of B. botulinus in the soil as the Mississippi Basin and the lower portion of its tributary, the Missouri. The

15 states which furnished less than 10% positive cultures belong topographically to the Great Plains. The average altitudes of these states from which samples have been collected are between 1,000 to 2,000 ft. It is impossible to connect the limited data thus far collected with any geological formation. Most of the soil samples belong to the class of residual rock. Future studies must furnish the solution of the many questions which these observations suggest, but it is believed that the low B. botulinus index in the Great Plains and the Mississippi Valley is, in the light of the observations made in California, mainly governed by the absence of heavily infected virgin mountain areas.

Six of the 15 North and South Atlantic States supplied soil and vegetables which contained B. botulinus in from 20 to 50% of the cultures, while those derived from 4 states, namely, Vermont, New Hampshire, Massachusetts and Connecticut, were positive in less than 10%, usually 5%, of the tests. These two extremes are balanced by the findings in the states of Florida, North and South Carolina, New Jersey and New York, which furnished between 10 to 20% typical cultures. As a whole, 15 Atlantic States, represented by 421 specimens, gave 96, or 22.8%, toxic and 71, or 16.9%, typable cultures, which were definitely proved to contain B. botulinus. This organism is, therefore, somewhat more frequently encountered in the Atlantic States than in those of the Great Plains and the Mississippi Valley. Pennsylvania and Maryland are exceptions, but it is not unlikely that the high percentage of positive cultures is the result of chance sampling or is dependent on the same soil stratum in both states, namely, a part of the Blue Ridge Mountain soil which supplied the samples. The relatively frequent findings of B. botulinus spores in the draining areas of these mountains, namely, New Jersey, Delaware and Maryland, lends considerable support to the last mentioned view and suggests the Blue Ridge Mountains as a breeding place for the neighboring valleys and river beds.

The number of examinations made on material supplied from the 4 North Atlantic States which gave less than 10% positive cultures is too small to justify any definite conclusions. The states are surrounded on both sides by soil stratums which harbor B. botulinus spores in a fairly large percentage of specimens. Attention is called in this connection to the existence of the organism in virgin forest soil in Main and New York. It is therefore not unlikely that extended sampling in Vermont, New Hampshire, Massachusetts and Connecticut may radically change the aspect of the data thus far collected.

The soil and vegetable specimens obtained from the South Atlantic States, Georgia, North and South Carolina, originated in the interior, while those of Florida represent coast, as well as inland, collections. Georgia and South Carolina yielded 25 and 18.1% positive cultures; soil strata of the foothills of the Blue Ridge Mountains supplied the samples. In Florida, the differences between the coast and the interior are clearly shown by the absence of B. botulinus in the samples collected around Miami and the fairly high percentage of infected material procured from the neighborhood of Gainesville. This interesting comparison indicates that virgin soil on the Atlantic Coast may be free from the spores of B. botulinus and, furthermore, emphasizes the conclusion previously drawn that the findings made on one topographical section do not necessarily apply to the state as a whole.

The results reported for the Middle and Atlantic States are in striking contrast to those revealed by the specimens procured from the Western States. Ten states, exclusive of California, supplied 449 samples, with 126, or 28%, toxic and properly identified cultures. Topographically these states form a part of the Cordilleran system, and, with the exception of Colorado and Idaho, furnish a B. botulinus soil index of 20%, while in 3 states, Oregon, Nevada and Washington, it is more than 30%. In the last named state, every section was found to contain this anaerobe, and the detailed findings made in the Yakima Valley deserve brief consideration. The percentage figures shown in chart 2 suggest that B. botulinus is, as in California, an inhabitant of the virgin soil. It may be mere coincidence due to methods of sampling, but the data indicate that from the mountain ranges toward the valleys, following the descending course of the rivers, there is a progressive reduction in the infection index of the soil. For example, virgin mountain soil contained B. botulinus spores in 83% of instances, while earth collected in the adjacent Tieton and Yakima Valley sections gave 57.1 and 55.5% positive cultures. On the other hand, the Toppenish area, which is farther removed from the source of the Yakima River and its tributaries, supplied comparatively few infected soil specimens (11.7%). The soil is equally polluted in the vicinity of Yelm and the virgin soil along the eastern ledge of the Nisqually Glacier on Mount Rainier.

The demonstration and isolation of B. botulinus from virgin sage brush soil and uninhabited sections of Nevada forcibly support the conclusion that this organism is universally distributed in the Western States. Additional evidence was also secured by carefully sampling

virgin districts in 3 National Parks: Yellowstone, Glacier and Mount Rainier, as well as the region surrounding Pike's Peak. Twenty-six to 40% of the soil samples collected in these regions yielded positive cultures and frequent isolations of the anaerobe. B. botulinus has been located at altitudes around 11,000 ft. in soil layers of glacial origin and on an isolated island of the Yellowstone Lake and has, by repeated

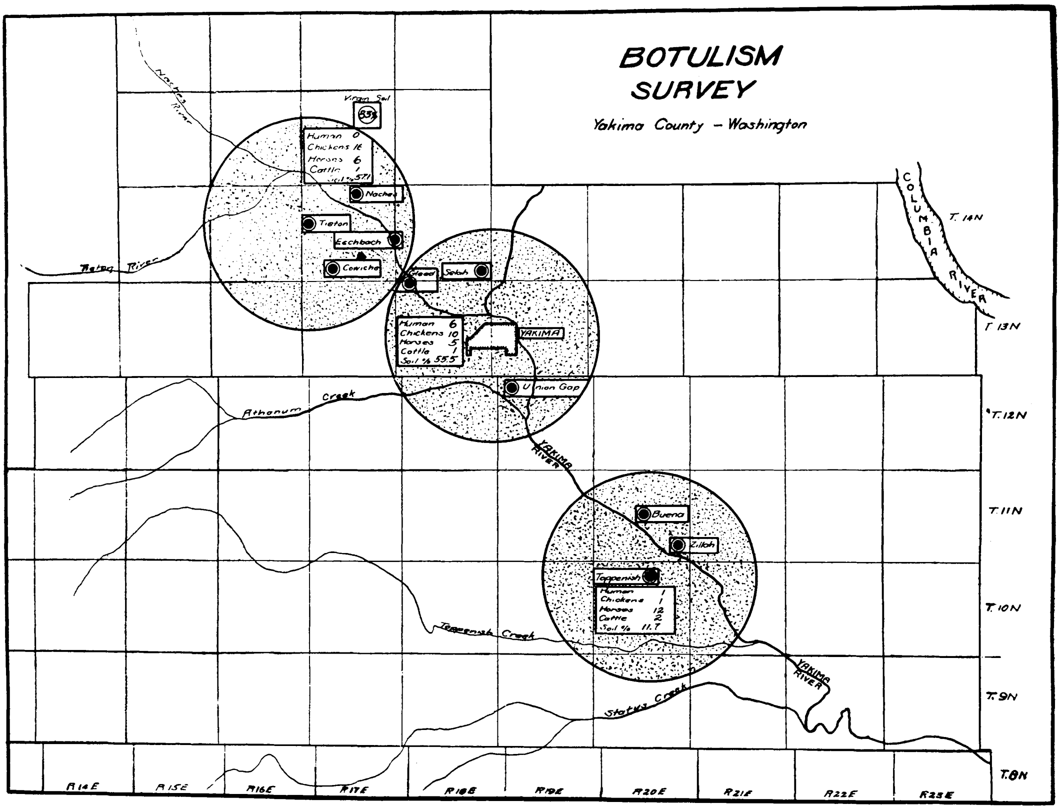

Chart 2

tests, been demonstrated in pure cultures. This evidence strongly refutes the conception of an intestinal habitat of the organism. Unfortunately little or no attention was paid to the geological structures which furnished the positive virgin soil specimens, and this survey does not permit an answer to the question: Can B. botulinus breed in these regions, and what are its biological functions? Work to solve these mysteries is in progress and will be reported when available.

Based on this survey, it must be concluded that B. botulinus is a common soil anaerobe of the Western States or the Cordilleran system; it is less frequently encountered in the Atlantic States and relatively rare in the Middle States, Great Plains and the Mississippi Valley.

(2) *The Distribution of the Two Types of B. botulinus.*—Through the early studies of Leuchs [2] and the recent work of Burke,[3] it is known that the antitoxin prepared against types A and B toxins is specific for the homologous toxin and will not protect against the heterologous one. This fact has been used to identify the toxin generated in the cultures prepared with the field specimens. A total of 256 cultures has been typed or identified by the isolation of B. botulinus in pure culture. It was recognized that certain states furnished only one type of B.

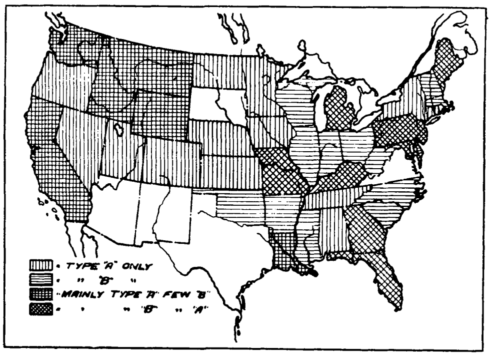

Chart 3

botulinus, namely, type A or B, while some gave, for example, predominantly type A, with one or two cultures yielding type B toxin, and vice versa. By grouping these data according to states and by using 4 contrasting shades, chart 3 has been obtained.

It is quite evident that the distribution of the 2 serologic types of B. botulinus divides the United States roughly into 2 distinct areas: The Western States, inclusive of the Great Plains, and the Mississippi Valley together with the Atlantic States. In a general way, the soil area spreading from the 95 degrees longitude westward to the 125 degrees longitude harbors mainly type A, while that extending from 95 degrees longitude eastward to the 65 degrees longitude supplies B. botulinus type B. The Mississippi Valley, with the valleys of its

[2] Ztschr. f. Hyg. u. Infektionskr., 1910, 65, p. 55.
[3] Jour. Bacteriol., 1919, 4, p. 555.

tributaries, the Ohio, the Red and a part of the Missouri, as well as the Great Lakes region, is characterized by a striking predominance of type B. Similarly prevalent is this type in the Atlantic States of Maryland, Delaware, New Jersey, Georgia, South Carolina, etc., while the scattered findings of type A, in Maine, New York and Pennsylvania indicate the probable existence of seed beds in the form of virgin forests and mountains. Alabama, Connecticut and Tennessee supplied only type A toxins, but it is not unlikely that more extensive sampling would also place these 2 states in the type B. area.

For several years, it was known that type A was prevalent in the Western States, and the data illustrated by chart 3 fully confirm the statement made in a former paper that this type is primarily an inhabitant of the virgin soil from which it may be scattered by the streams and rivers to distant regions, even invading the Missouri and Mississippi Valleys (see Missouri and Louisiana). In Colorado, Oregon, Nevada, Utah, North Dakota, Nebraska, etc., type A is the only type encountered, while in Washington, Idaho, Montana and Wyoming type B is occasionally found; in every instance, the specimens infected with the spores of the latter type were either soil or vegetables from gardens or forest or moraine soil rich in decayed plant material. One sample of moldy alfalfa hay from Bozeman, Mont., contained a mixture of type A and B. The facts so revealed agree perfectly with the data presented in a former paper, namely: Type B is probably an adaptation mutant to the physical and chemical influences encountered by type A in tilled and cultivated soil. Whether the same explanation applies to the conditions in the Middle and Atlantic States cannot be stated with certainty. It may be mere coincidence, but by comparing chart 3 with a recent map showing the density of population in the United States according to the 1920 census, it will be noted that the type B area is nearly covered by that holding a population of from 18 to 400 persons per square mile. In other words, the soils which are subjected to intensive cultivation and fertilization contain, as a rule, B. botulinus, type B. A similar condition apparently exists in Canada and on the European continent.

Further comparison of chart 3 with chart 1 indicates that the specimens derived from states which yielded only, or at least predominantly, type B, produced also a low percentage of toxic cultures. When type B is isolated, the number of spores in the soil and its products is relatively small. This interesting condition was first noted in California and has since then been confirmed by the studies made in other states of the

Union. These facts have an encouraging aspect from the standpoint of botulism prevention, namely, intensive agriculture may in generations suppress B. botulinus as a soil anaerobe and consequently eliminate the danger of spore contamination on vegetables, fruits and feeds, provided the rivers and streams draining heavily infected areas of type A cease to reinfect these districts. Extensive irrigation as practiced in California and Colorado has been and is probably to a great extent responsible for the pollution of the cultivated fields and orchards with highly heat resistant spores of B. botulinus, by nature only present in the mountain soil. The significance of type B in its relation to human botulism is discussed in a subsequent paragraph.

(3) *The Telluric Distribution of B. botulinus.*—In chart 4, the cultural results of the 1,538 field specimens are arranged in graphic form according to their telluric origin, namely, virgin, cultivated, garden and pasture soil, dirt from animal corrals and vegetables and feeds. This presentation emphasizes the predominance of B. botulinus in virgin and pasture soil and the relatively low percentage of positive cultures in soil and manure collected from animal corrals, pig pens and chicken houses. As a whole, the data correspond closely to those already mentioned for California. Earth contaminated with manure or animal excreta rarely carries the spores of B. botulinus. In fact, fertilization cannot be considered a factor contributing to the pollution of the soil. Moreover, these columns are responsible for the suggestions made in the preceding paragraphs, namely, cultivation and cropping of the soil reduces or dilutes the number of spores to such a degree that the methods employed for detection fail to demonstrate their presence.

This view is also supported by the examination of soil products. Soil stratums furnishing nontoxic or weakly toxic cultures frequently supplied vegetables or feeds, which contained B. botulinus, type B. It is possible that the concomitant anaerobes or aerobes of the cultivated or manured soil inhibit the growth or the toxin production of the few spores of B. botulinus present in the usual amounts chosen for these tests. As a rule, the vegetable samples examined have been less heavily polluted with various antagonistic soil bacteria and demonstration of the toxicogenic anaerobe has been more readily accomplished than with soil specimens. In any case, 1 gm. of cultivated or manured garden soil contains quantitatively fewer spores than the same amount of virgin soil.

The cultural findings made on vegetables and feeds reflect the flora of the soil and may indicate the potential danger from B. botulinus

in a community. In this connection, it should, however, be emphasized that the mere demonstration of the organism in feeds does not signify that this material has in the past or may in the future cause animal

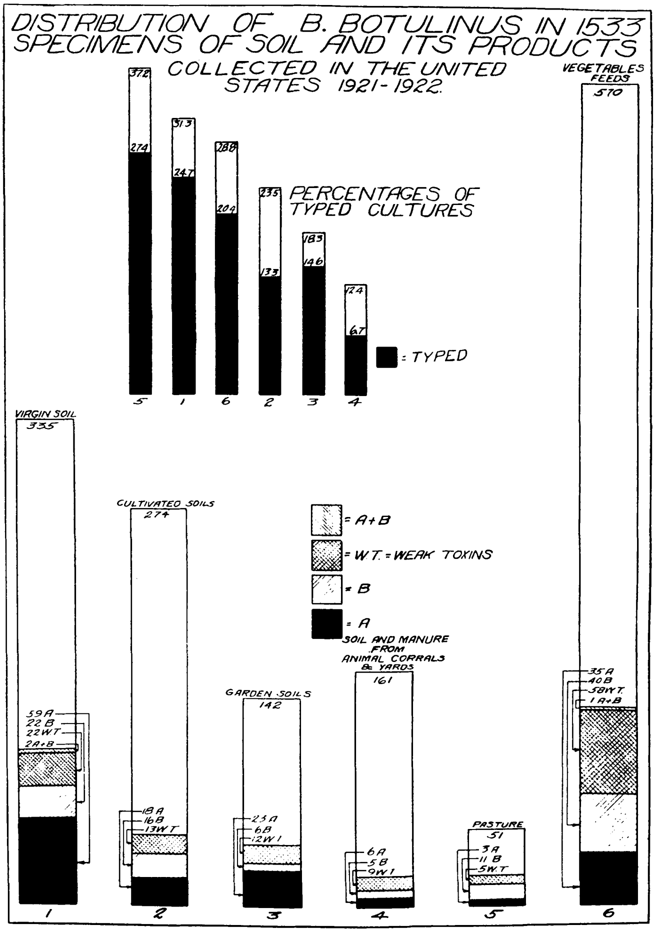

Chart 4

botulism. A number of workers dealing with certain animal diseases in the Middle Western States have repeatedly failed to appreciate these facts, and they have invariably made the diagnosis of botulism provided they were able to isolate B. botulinus from the suspected feed or the spleens and livers of dead horses, swine and sheep.

In table 2, the various vegetables, fruits and feeds which have been examined are tabulated and the percentages of positive cultures have been calculated for each important plant material. There is no doubt that every soil product may sometimes carry the spores of B. botulinus, but in the light of the epidemiology of botulism in this country, it is significant that string beans and leaves furnished the highest percentage of toxic and identified cultures. In this connection, it should be recalled that 45 instances are on record in which home or commercially packed string beans caused outbreaks of human (20) and avian (25 botulism. The factors responsible for the frequent contamination of string bean pods or vines deserve more careful experimental investigation, not

TABLE 2

PERCENTAGE OF POSITIVE CULTURES IN VARIOUS VEGETABLES, FRUITS AND FEEDS

Vegetables and Feeds	Total Number Examined	Number of Positive Cultures	Percent of Positive Cultures
Cornhusks, leaves and stalks	80	6	7.5
Peapods, stalks and leaves	51	3	5.4
Moldy hay	44	7	15.9
String beans, pods and stalks	44	14	32.7
Beets, roots and tops	37	6	16.2
Decayed vegetation	29	6	20.6
Tomato plant and roots	24	2	8.3
Lettuce	21	3	14.2
Carrots	18	2	11.1
Turnips	18	2	11.1
Cabbage	16	3	18.7
Ensilage	15	3	20.0
Radishes	15	1	6.6
Decayed straw	13	1	7.6
Straw	10	0	....
Bean roots	10	1	10.0
Spinach	10	0	....
Lima beans	9	1	11.1
Cucumber root	8	0	....
Pumpkin vines	8	0	....
Sweet potatoes	8	1	12.5
Swiss chard	8	0	....
Potatoes	8	2	25.0
Onions	8	1	12.5
Forage	6	2	33.3
Alfalfa	4	1	25.0
Parsnips	4	0	....
Asparagus	4	1	25.0
Oats	4	1	25.0
Parsley	4	0	....
Soya beans	4	1	25.0
Cherries	3	0	....
Dandelions	2	1	....
Hominy	2	0	....
Beet pulp	2	0	....
Mango tops	2	0	....
Red pepper	2	0	....
Mustard	2	0	....
Daffodils, cornfodder, wheat head, squash, crabapple, rutabaga, moldy barley, canna tops, cauliflower, curari roots, clover, strawberries (one each)	13	3	....
Total 50 soil products	570	75	

only from a biologic but also from a preventive standpoint. Plant material undergoing decay and fermentation, as for example vegetables, moldy hay and ensilage, supplied a high percentage of toxic cultures and isolations of B. botulinus. According to the reports accompanying the shipments of these samples, it was known that some of the moldy hay and ensilage containing B. botulinus had been consumed by horses and cattle with impunity. A few toxicologic examinations conducted in this laboratory failed to demonstrate the presence of B. botulinus toxins in these samples. These and similar observations cast serious doubt on the majority of claims which in recent years associate any mysterious animal disease with botulism. In fact, it is evident from the data presented in table 2 that B. botulinus is frequently found in moldy hay and ensilage but, so long as these feeds are free from toxin, they may be ingested by herbivorous animals without causing botulism.

From a biologic standpoint, it is by no means surprising to find B. botulinus frequently associated with decaying plant material. The organism, being a spore-bearer, rarely meets in nature the conditions suitable for its growth, and its continued existence would be impossible without some resting stage. In soil, conditions favorable to the growth probably occur when decaying vegetation furnishes the necessary food material. These conditions probably develop just often enough so that the spores do not diminish in number. This behavior deserves further study, but this possibility should be fully appreciated in canneries and utmost care exercised in the disposal of waste products. It is not unlikely that the most resistant spores of B. botulinus are not introduced into the packing plant on the fresh and sound vegetables and fruits, but are made available under unsanitary conditions of waste disposal. The principles which apply to the suppression of heat resistant thermophiles can unreservedly be used in the control of B. botulinus spores.

A total of 50 specimens of beans, cucumber and tomato roots, not tabulated, revealed only one positive culture, while the vines and leaves of the same plants from the same source furnished 10 or 20% positive cultures. These and similar tests should be made on a larger series of samples, but they confirm in a general way the previously made observations that B. botulinus occurs more often in the surface layers of the soil and consequently can cause dust borne contaminations of sound fruits on the trees. This condition has repeatedly been noted in the olive orchards of California.

TABLE 3

NUMBER OF OUTBREAKS OF BOTULISM COMPARED WITH PERCENTAGE FIGURES OF SOIL AND VEGETABLE SPECIMENS CONTAINING SPORES OF B. BOTULINUS

State	Botulism Outbreaks						Percent of Specimens Containing B. botulinus Spores			
	Human				Fowl: Locally Grown, Home Packed Food Products	Horse and Mules: Locally Grown Feed, etc.				
	Total	Locally Grown, Commercially Packed	Locally Grown, Home Packed or Preserved	Unknown			Typed	Type A	Type B	Type A and B
Arizona	1	(1 Ohio)	..	..	..	..	....	.....	.....	.....
California	47	11 (1 Ohio)	29	6	53	?	**22.2**	**78.4**	22.1	3.1
Colorado	3	1 (?)	1	..	3	(1)	**17.4**	**100.0**	.....	....
Dist. Columbia	..	..	..	..	1	..	....	.....	.....	.....
Florida	1	..	1	..	..	..	17.7	12.5	87.5	.....
Idaho	3	..	3	..	..	..	12.1	**75.0**	25.0	.....
Illinois	1 (?)	1 (?)	..	..	1	1	5.1	.....	100.0	.....
Indiana	3	.. (2 California)	1	..	..	..	17.8	.....	100.0	.....
Iowa	1(?)	..	1(?)	..	..	..	2.9	100.0	.....	.....
Kansas	..	1 (?) (Colorado)	..	..	..	..	6.6	100.0	.....	.....
Kentucky	..	..	..	..	..	1	70.5	.....	100.0	.....
Maine	1	..	1	..	..	..	**24.3**	80.0	20.0	.....
Massachusetts	3	..	2	1	..	..	5.8	.....	100.0	.....
Michigan	3	0 (3 California)	..	..	..	..	4.1	20.0	80.0	.....
Montana	1	0 (1 California)	..	..	..	..	24.2	75.0	18.7	6.3
Nevada	..	..	..	..	5	..	**36.0**	100.0	.....	.....
New Jersey	1	..	1	..	..	..	19.4	14.3	85.7	.....
New York	5	0	3	1	..	..	**19.3**	**100.0**	.....	.....
Ohio	2	3 (California, Arizona, W. Virgina) (2 California)	..	..	..	..	7.3	.....	100.0	.....
Oregon	4	0	4	..	2	..	**34.2**	**100.0**	.....	.....
Pennsylvania	2	0 (1 California)	1	..	..	..	**35.0**	40.0	60.0	.....
Tennessee	1	0 (1 California)	..	..	..	..	15.0	100.0	.....	.....
Texas	1	1	..	..	..	..	0	0	.....	.....
Washington	14	1	12	1	23	..	37.8	**89.5**	11.4	.....
West Virginia	1	0 (1 Ohio)	..	..	..	..	4.8	.....	100.0	.....
Wisconsin	1	1	..	..	..	..	8.3	.....	100.0	.....
Wyoming	1	..	1	..	..	..	**25.0**	**84.7**	15.3	.....
Total 27 states	102	32	61	9	88	3				

(4) *Relation of Distribution of B. botulinus in the Soil and Its Products to Outbreaks of Human and Animal Botulism.*—In table 3, the number of botulism outbreaks thus far recognized in the different states is compared with the percentage figures of soil and vegetable specimens found to contain the spores of B. botulinus.

It is not surprising to note that when the percentage figures of positive cultures exceed 20 or 30%, and particularly when type A predominates, human and animal botulism is not infrequent. California, Colorado, Idaho, Maine, Nevada, New York, Oregon, Washington and Wyoming are excellent examples of these conditions. The states furnishing B. botulinus, type B either report no outbreaks, or botulism is caused by food products which have not been preserved by heat (sausages, hams, etc.). Comparative studies have shown that the spores of the majority of B. botulinus, type B strains are, as a rule, less heat resistant than those of type A (average of 23 strains type B, at 105 C. = 37 minutes and of 33 strains type A, at 105 C. = 62 minutes). It is, therefore, not unlikely that the absence of botulism in the states, which primarily harbor type B is due to the relative scarcity and possibly the low heat resistance of the prevailing spores of B. botulinus. This explanation is based on the strains thus far available, but it should be stated with utmost emphasis that spores of type B have been grown in this laboratory which have withstood a temperature of 105 C. for at least 65 minutes (strains 13, 41, 77 and 63) or the average resistance of type A strains.

No assurance can be given that such spores may not occasionally enter the food product to be preserved, and it would be most unwise to disregard in a Middle Western or Eastern state vigilance and rigid procedures of sterilization merely on account of the conditions indicated by this survey. From a practical standpoint, the spores of B. botulinus are ubiquitous; they may be found anywhere and any time, although certain locality variations deserve the attention of the biologist.

Outbreaks of fowl botulism are usually due to preserved vegetable products, and the same factors which lead to human cases are responsible for these fatalities. In fact, an approximate estimate of spoilage due to B. botulinus can be made from the various outbreaks of botulism among barnyard fowls, which developed symptoms of the disease or died following the consumption of poisonous, spoiled food. It is to be regretted, therefore, that reliable epidemiologic data relative to the number of outbreaks of botulism among fowls in states other than California and Washington are not available. On observation, in which

spoiled corn containing B. botulinus type B was the cause, has been reported from Washington, D. C. In another, which occurred in New Jersey, the type of the organism was not determined; while B. botulinus type A was found incidentally in a sample of chicken manure collected at East Lansing. By analogy with human outbreaks, it must be suspected that localities yielding a high percentage of B. botulinus in soil will furnish outbreaks of avian botulism. This statement deserves additional investigation and is made with reserve, as dependable data are not available. The same must be said with regard to the so-called outbreaks of equine, bovine and porcine botulism. In California, where every opprotunity has been selected to verify the clinical diagnosis of equine botulism by careful laboratory tests, it has been impossible to find even one instance in which the suspected moldy hay, corn or straw contained the toxin of B. botulinus. The bacteriologic demonstration of B. botulinus in the tissues of horses and of cattle cannot be used as a criterion or as proof that certain forms of "forage poisoning" are actually botulism. Laboratory findings also indicate that this anaerobe may sometimes be demonstrated in the organs of animals which die of other diseases than botulism. Furthermore, states with a relatively low B. botulinus soil index report extensive outbreaks of "forage poisoning," and vice versa. In the light of these conditions, it is obviously impossible to draw deductions which can demonstrate any relationship between spore prevalence and equine or bovine botulism.

CONCLUSIONS

A general survey, during which 1,538 soil, vegetable, feed and manure specimens of every state of the United States, except Virginia, have been studied for the presence of the spores of B. botulinus, reveals the following facts:

1. B. botulinus is a common soil anaerobe of the Western States of the Cordilleran system. It is less frequently encountered in the Atlantic States and is relatively rare in the Middle States, the Great Plains and the Mississippi Valley.

2. The soil of the Western States, inclusive of the Great Plains, yield, mainly, B. botulinus, type A, while the Mississippi Valley and Great Lakes region is characterized by a striking predominance of type B. Similarly prevalent is this latter type in the Atlantic States of Maryland, Delaware, New Jersey, Georgia and South Carolina, while scattered findings of type A in Maine, New York and Pennsyl-

vania indicate the existence of breeding places in virgin forests and mountains. Soils which are subjected to intensive cultivation and fertilization contain, as a rule, B. botulinus, type B.

3. B. botulinus spores are far more prevalent in virgin and pasture soils than in dirt, soil or manure collected from animal corrals, pig pens, etc. Vegetables, fruits and feeds are frequently contaminated with the spores of B. botulinus. String bean pods and leaves, moldy hay, ensilage and decayed vegetation may yield a relatively high percentage of positive cultures.

4. Human and animal botulism is not infrequent in those states in which B. botulinus, type A, predominates, or in which the percentage figures of positive cultures exceeds 20 to 30%. From a practical standpoint, however, B. botulinus is ubiquitous, and this survey gives no assurance that heat resistant spores cannot be found anywhere and at any time.

5. The theory which claims that all the pathogenic anaerobes are regular inhabitants of the intestinal canal of animals deserves renewed investigation in the light of this survey on B. botulinus.